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OCT 16 1953

VOLUME 11 NUMBER 10

NUTRITION REVIEWS

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October 1953

Special Article

Early Experiences with Niacin—A Retrospect 289

Clinical Nutrition

Overweight and Obesity as a Medical Hazard 292
The Renal Clearance of Pantothenic Acid 295
Blood Copper in Man 296
Fructose and Invert Sugar Versus Glucose 299
Lean Body Mass as a Metabolic Reference Standard 301

Experimental Nutrition

The Effect of Vitamin B_{12} on Sulfhydryl Compounds 304
Functional Vascular Defects in Experimental Scurvy 306
Modification of the Effects of Cholesterol Feeding in the Chick .. 307
Fat Deficiency and Endocrine Function 308
Factors Influencing the Level of Free Choline in Plasma 309
Relative Humidity and Nutritional Pathology 311
Metabolic Blocks in Diabetes 312
The Action of Vitamin B_6 Antagonists in Microorganisms 314
The Diabetogenic Activity of Pituitary Extracts 315
Antibacterials and Growth 317

Notes

Letter to the Editor 319
American Board of Nutrition 319
Nutrition for Airmen and Industrial Workers 319
Milk Diets and Vitamin B_{12} 319
Clinical Nutrition Briefs 320

Published Monthly by

THE NUTRITION FOUNDATION, INC.

CHRYSLER BUILDING NEW YORK 17, N. Y.

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NUTRITION REVIEWS

VOL. 11 OCTOBER 1953 No. 10

EARLY EXPERIENCES WITH NIACIN—A RETROSPECT

Several people looking into the same kaleidoscope will see the identical completed designs, but to each, different colors and shapes in the pattern will stand out. So it is when different workers attempt to look in retrospect at research in which they have had a part. A discussion of the early work on niacin is especially difficult because it is so closely interwoven with the developments in the entire field of the vitamin B-complex.

I could not have grown up in the Department of Agricultural Chemistry at the University of Wisconsin without having a real interest in vitamins and minerals. The seminars conducted by Professor E. B. Hart not only gave us an interesting background to the entire field of nutrition, but stimulated us to new thinking and new ideas. Naturally the B-vitamins were discussed, but relatively little active work was in progress in the department on this subject when I started as a graduate student. However, one problem which did interest Professor Hart a great deal was the nutritional difference between a diet composed entirely of vegetable matter and one containing some animal products. Incidentally, the recent work on vitamin B_{12} has solved the question to a large extent. As a result of these questions chicks were placed on a diet made up of yellow corn, wheat middlings, casein, salt, cod liver oil and calcium carbonate. It was found that a vitamin B_1 deficiency could be produced by autoclaving this ration and another deficiency which we called vitamin B_2 deficiency was obtained by heating this ration in a dry condition (O. L. Kline, J. A. Keenan, C. A. Elvehjem, and E. B. Hart, *J. Biol. Chem.* **99,** *295 (1932)*). Vitamin B_2 had been considered the more heat-stable constituent of the vitamin B-complex since the early work of Goldberger, Wheeler, Lillie and Rogers in 1926.

The symptoms observed in the chicks on the heated ration were similar to those described by Norris and Ringrose in 1930, but later work has shown that several B-vitamins are involved in the prevention of dermatitis in this species. This early work was in conjunction with Professor Hart and with O. L. Kline and J. A. Keenan, who were graduate students at that time. I had received my PH.D. degree in 1927, but continued as an instructor in the department during the next two years. During 1929–1930 I worked at the Sir William Dunn Institute in Cambridge, England, and when I returned to the department in the fall of 1930, the first graduate student to work with me was Fred Stare. I had brought back with me from England a Barcroft respirometer and Fred and I started working on tissue metabolism. We hoped to be able to show certain distinct changes in tissue metabolism due to vitamin deficiencies.

The second student to work with me was Carl J. Koehn, Jr., who had excellent training in basic chemistry. Since we had a method available for assaying vitamin B_2 (pellagra factor) we turned our attention to isolation of this factor. We used commercial liver extract (pernicious anemia fraction) because we had used this material in anemia studies and because Goldberger and Sebrell had shown it to be effective in canine blacktongue and Spies had used it in human pellagra. In 1934 we obtained a concentrate which showed a distinct greenish fluorescence and which was highly active in the prevention of the so-called pellagra in chicks. In the meantime German workers had isolated flavins from egg white, whey, liver and yeast and had suggested that they were active as vitamin B_2. However, we found that the flavin was adsorbed on fuller's earth in acid solution and that the factor preventing pellagra in chicks remained in

the filtrate. The fluorescence due to the flavins in concentrates from liver was destroyed by exposure to sunlight or artificial light without altering the activity for the chicken (C. A. Elvehjem and C. J. Koehn, *J. Biol. Chem.* **108,** *709 (1935)*). Thus the factor necessary for the prevention of pellagra in chicks was a compound separate and distinct from what we know today as riboflavin.

The work with chicks became more involved and at one point Professor Hart suggested that we stop working on the B-complex since it was too complicated. I replied that it is when things look most complicated that the greatest progress may be made. The work with chicks continued and as is well known today the chick proved very useful in elucidating many of the members of the B-complex.

However, since Koehn and I were interested in the pellagra factor we decided it was necessary to turn to the use of dogs. We tried to compound a diet similar to diet number 323 of Goldberger, but we had difficulty in obtaining cowpeas. Nevertheless we produced typical symptoms of blacktongue in dogs by using a modified Goldberger ration without cowpeas. Preparations of hepatoflavin from liver were found to be completely inactive in the prevention or cure of blacktongue. Preparations from liver extract from which the hepatoflavin had been removed were found to be highly active. These observations were reported in the Journal of Nutrition in the early part of 1936.

Studies on the concentration of the antipellagra factor were continued and at the meeting of the American Chemical Society in Pittsburgh, September 1936, we reported that we had obtained 2.56 g. of active dry material from 400 g. of liver extract and that 64 mg. of this material completely cured a dog of blacktongue. Then as so frequently happens with graduate students Carl became Dr. Koehn and accepted a position at Auburn, Alabama. During the summer of 1936, several new students arrived, including A. E. Axelrod, Douglas V. Frost and Robert J. Madden. The isolation work was continued by Madden, but the assays were slow and tedious since it took many weeks to produce typical blacktongue in the dogs. As more highly active concentrates were prepared we called upon others in the department who had had more experience in isolation work. These included Dr. Strong, who was a member of the department, and Wayne Woolley, who was working with Professor W. H. Peterson. Little success in obtaining a crystalline material resulted until our concentrates were passed through a molecular still. When crystals were finally obtained and it was necessary to study the elemental composition we were fortunate in having the help of Mr. H. A. Campbell and Dr. K. P. Link. After certain difficulties in final analysis the crystals were identified as nicotinic acid amide. The actual identification of the nicotinamide was made on 58 mg. from an original batch of 175 mg. of long white needles obtained from liver.

However, we had not limited all of our work to dogs and even before Carl Koehn left the laboratory, attempts were made to use purified rations with rats to study these newer factors. Jerry Olson had worked with us and in October 1936 we had published a paper on a new essential dietary factor which seemed to be distinct from the then known dietary essentials. This work had been carried on by Douglas Frost and we had used the simple term "factor W" to designate this factor. Since nicotinic acid had shown some growth-stimulating effects in rats reared on these purified diets, and since Warburg and von Euler had shown it to be a constituent of coenzyme I, we decided to test nicotinic acid itself on dogs. A dog showing all the symptoms of blacktongue was given a single dose of 30 mg. of nicotinic acid on July 10, 1937 and a phenomenal response was obtained. This then was probably the first response ever

recorded in which nicotinic acid showed a relationship to blacktongue. Since the crystals had been identified as nicotinic acid amide, nicotinic acid amide was prepared in pure form and it too was found to be active in the dog. A preliminary report of this work was published in the Journal of the American Chemical Society in the fall of 1937. The complete report was not published until March 1938, and by that time numerous cases of human pellagra had been treated with nicotinic acid.

The developments now came rapidly in all directions. The early work which we did on tissue metabolism was now of real value in understanding the function of nicotinic acid in living tissues. The metabolism work started with Stare was continued by Van Potter and Morris Lipton and a little later by Axelrod. Axelrod was immediately ready to study changes in the coenzyme content of tissues as the result of nicotinic acid deficiency (A. E. Axelrod, R. J. Madden, and C. A. Elvehjem, *J. Biol. Chem.* **131,** *85 (1939)*). The method which Axelrod developed for coenzyme I in 1939 has been used extensively since that time.

Drs. Strong and Woolley were interested in studying the antiblacktongue activity of various pyridine derivatives. In August 1938 the activity of a large list of compounds was published. It may be interesting to point out that in this paper it was suggested that beta-acetyl-pyridine and pyridine-3-sulfonic acid seemed to be toxic to dogs suffering from blacktongue deficiency. These compounds did not prove toxic to normal dogs when fed at similar levels. This observation later led Dr. Woolley to inaugurate the newer concept of antimetabolites.

The chicken was not forgotten in the later studies and in the meantime Harry Waisman and Olaf Mickelsen had extended the studies on the chick and showed that nicotinic acid was not active in preventing the so-called chick pellagra (O. Mickelsen, H. Waisman, and C. A. Elvehjem, *J. Biol. Chem.* **124,** *313 (1938)*). A little later these workers, in cooperation with Dr. Woolley, made considerable progress in isolating the factor which later was shown to be pantothenic acid. Although the availability of nicotinic acid for purified rations for chicks and rats was of no immediate value, the fact that another B-vitamin had been characterized stimulated work on additional factors. The availability of nicotinic acid for synthetic diets for dogs was of real value and did allow more extensive use of the dog for studies on vitamin B_6 and the factors which are today known as folacin and vitamin B_{12}.

It is of course impossible to look back on the early work on nicotinic acid without mentioning why we encountered difficulty in producing this deficiency in rats and chicks. This has now been completely clarified due to the observations on the relation of nicotinic acid to tryptophan. I might mention that the observation was the result of curtailment of our studies on human subjects. When milk was found to be low in niacin, we wondered where the young infant received his supply. Arrangements had been made with Dr. John Gonce of our Department of Pediatrics to use young infants in balance studies when World War II started, and prevented us from carrying out this program. As a result, Bill Krehl put rats on a typical pellagra-producing diet and as a result of this it was found that both niacin and tryptophan would prevent the reduced growth on the corn diets. The developments have been so extensive in this relationship that it is impossible to mention them in any detail. However, I do wish to point out that all of the work has completely verified the original observations of Dr. Goldberger, and although he did not live to see the developments, it was my pleasure a number of years ago to point them out to Mrs. Goldberger. It is also interesting that very recently Dr. W. H. Sebrell referred to a letter on file at the National Institute of Health, written by W. F. Tanner to Dr.

Goldberger, dated August 5, 1921, which included the following statement about a patient treated with tryptophan, "... the improvement in this patient has surpassed anything I have ever seen in a case of pellagra in an equal period of time."

It gives one a great deal of personal satisfaction to reflect upon these interesting developments, but to me they have much greater implications. It proves without doubt that if young research workers are given a few facilities and some encouragement, the intricacies of living matter can be unraveled. The part played by niacin has unfolded slowly but logically. The pieces of the jigsaw puzzle fit together very nicely, although there are still areas upon which to work. The important contributions were made by young workers who have continued their interest in related fields of nutrition. Finally, it is an excellent example of willing cooperation between workers which must be the basis of future research, whether in nutrition or any other field of research.

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OVERWEIGHT AND OBESITY AS A MEDICAL HAZARD

D. P. Barr refers to obesity as a "red light of health" ("Overeating, Overweight and Obesity," *Nutrition Symposium No. 6, 90, National Vitamin Foundation, New York (1953)*), a stop sign which should be a warning to the prospective victims and should be considered as such by the medical profession. However, it is not from a medical treatise but from W. Banting's "Letter on Corpulence" (*Harrison, London (1863)*) that he takes his forceful motto: "Of all the parasites that affect humanity I do not know of nor can I imagine any more distressing than that of obesity." This will be granted readily for a grotesque degree of overweight which is esthetically unappealing and in which every physical activity is a burden, with shortness of breath on moderate exertion, excessive sweating, and intolerance of heat.

It is the insurance statistics as well as experimental and clinical studies which provide data indicating that even less marked degrees of overweight represent a threat to continued good health and longevity in the middle and later years of life.

E. C. Bonnet, medical director of the Metropolitan Life Insurance Company (*Tr. Assn. Life Insurance Medical Directors of America* **35,** *263 (1952)*), expressed the opinion that overweight is perhaps the most common impairment which confronts the life insurance underwriter. However, the existing studies among insured overweights are not without flaws and the need was stressed for a study concerned with excess mortality because of underweight alone, not complicated by wide variations in the type of medical examination on the basis of which a life insurance policy is issued. He pointed out that in the study on mortality among insured overweights reported by L. I. Dublin and H. H. Marks (*Ibid., p. 235*; *Nutrition Reviews* **11,** *144 (1953)*), no uniform procedures had been applied. In the case of individuals 45 years of age or less applying for not more than a $2000 policy, no blood pressure measurement or urinalysis was required. Such individuals constituted a large percentage of the substandard policies issued during the years included in the study of Dublin and Marks, and a part of the excess mortality observed in their study may be an artifact. These individuals could have had high blood pressure, nephritis, or diabetes at the time of the application for life insurance. Furthermore, the weight was ordinarily *estimated*, not measured, by the examiner, and it appears that many of these

individuals have received lower overweight ratings than their actual weight would have called for. These facts represent serious methodologic limitations, and a call for a new study of the excess mortality because of *overweight alone* is fully justified. Such a study is needed as a guide for the underwriting of such risks in the future, as well as a means for a valid estimate of the medical hazards of overweight. The cases used for the study should be selected from a common baseline, including physical examination (with measured height and weight), blood pressure measurement, and urinalysis.

Separate papers, presented at the sixtieth annual meeting of the Association of Life Insurance Medical Directors of America, were devoted to the three leading causes of high mortality among overweight individuals: diabetes, hypertension, and atherosclerosis. E. S. Dillon and J. M. Trapnell (*Tr. Assn. Life Insurance Medical Directors of America* **35,** *280 (1952)*) summarized clinical and insurance statistics on the association of overweight with diabetes. In E. P. Joslin's study of 1000 cases of true diabetes, 71 to 89 per cent of adults (age 21 and up by decades) were 5 per cent or more above the standard weight ("The Treatment of Diabetes Mellitus," *8th ed., Lea and Febiger, Philadelphia (1946)*) and the tendency toward diabetes among the obese is confirmed by a wealth of other data. However, the part played by overweight in *producing* the disease is not readily apparent. There is no direct causality or a perfect correlation, as only a fraction of overweight individuals develops diabetes. On the other hand, the disease may be found in habitually underweight adults.

The hereditary tendency is well documented both for obesity and diabetes. The data on increased frequency of occurrence of the disease among blood relatives of persons suffering from diabetes do not help to clarify the underlying mechanisms and the role of obesity in the etiology of diabetes. The defect is endocrine in origin (faulty activity of the insulin-producing islets of Langerhans in the pancreas). The authors, leaning on C. N. H. Long (*Lancet* **II,** *106 (1951)*), believe that in individuals indulging in overeating a chronically large demand on the pancreas to supply insulin eventually overtaxes its capacity and brings on diabetes. The illness is considered to be at first reversible by weight reduction, but with increasing duration of obesity it tends to become established permanently. In contrast to adults, children who develop diabetes tend to be of normal weight or underweight. It is assumed that a child who inherited an inadequate pancreas may develop frank diabetes under the strain of rapid growth resulting from an overactive pituitary gland.

Dillon (*loc. cit., p. 286*) noted that the leading cause of mortality among diabetics is no longer severe acidosis and coma, but atherosclerosis and other degenerative lesions. This complicating disease develops early in diabetic patients, and progresses rapidly, affecting especially the coronary arteries and the arteries of the lower extremities. Dillon feels strongly that the control of diabetes (and the control of body weight) is an important factor in prevention of atherosclerosis. The matter of the fat content of the diet was not regarded as crucial, but no satisfactory evidence was offered on this point.

The dietary factors in the development of atherosclerosis, one of the major health problems in the United States, were considered by T. M. Durant (*Tr. Assn. Life Insurance Medical Directors of America* **35,** *267 (1952)*; *Ann. Int. Med.* **35,** *397 (1951)*). He pointed out that the geographic incidence of the disease points toward an apparent relationship between fat intake and atherosclerosis. The diminution of coronary artery accidents observed during World War II in countries with reduced intake of fats seems to point in the same direction, even though the evidence is less reliable. Disturbance of lipid metabolism, in disease and in experi-

mental animals, predisposes to atherosclerotic disease, but the metabolic theory of atherosclerosis has to face such facts as the one that not all persons with coronary artery disease have an elevated blood cholesterol level. It is not yet clear whether our understanding of this subject has been helped or further confused by reference to lipid molecules of different flotation rates, as determined by the ultracentrifuge.

In the discussion of the dietary factors and atherosclerosis, Durant's analysis (on the basis of the Department of Agriculture statistics) of the per capita consumption of food in the United States during 1910 and 1948 is of interest. In 1910 in the average American diet, protein contributed about 12 per cent, fat 32 per cent, and carbohydrates 56 per cent of the total caloric intake. In 1948, the protein figure remained unaltered while the fat percentage increased (to 39 per cent) and carbohydrates decreased (49 per cent). Of particular interest are the data on the diet of the business and professional men, a group noted for a tendency toward coronary disease, for whom the calculations based on the author's data yield a figure of 58 per cent of the calories being derived from fat. Their diet contrasts strikingly with the therapeutic regimen in use in the treatment of coronary disease in which the three basic types of food—protein, fat, and carbohydrates—provide 16, 14, and 70 per cent of the calories. Even should the data on the business and professional men prove to be an overestimate, there is little doubt that the *average* American diet has a very high fat content and that this fraction of the caloric intake has been rising steadily during the past fifty years.

As in diabetes, the relation between obesity and atherosclerosis seems to be complicated by the age factor. S. M. Garn, M. M. Gertler, S. A. Levine, and P. D. White (*Ann. Int. Med.* **34,** *1416 (1951)*) observed that in 97 men who had experienced myocardial infarction prior to the age of 40, obesity was not a characteristic feature. While the sample was small, the findings do furnish evidence that nonobese individuals may be victims of early atherosclerotic disease. Durant's experience (*loc. cit.*) was in accord with the findings of Garn *et al.* in that it is not unusual to see young coronary patients who are of normal weight, or even somewhat underweight. He interprets these findings by postulating inherited defects in metabolic machinery severe enough to result in an early and serious coronary involvement, without requiring a very large fat and caloric intake which would account for a disturbance of lipid (cholesterol) metabolism and result in obesity as well. After the age of 40, obesity becomes a much more common accompaniment of coronary disease.

Obesity is clearly a result of high caloric intake, coupled with a declining physical activity during the period of maturity. It is also very likely that there are qualitative differences in the diet. Actually, as far as the reviewer is aware, no comprehensive study of the diet consumed by obese individuals is available. This seems to be an important missing link in the argument about the role of dietary fat in the etiology of atherosclerotic disease.

The mechanism by which obesity facilitates the development of atherosclerosis was considered by J. W. Gofman and H. B. Jones (*Circulation* **5,** *514 (1952)*). Using a deviation from "normal" weight as a crude criterion of obesity (*i.e.*, "fatness"), they obtained weak but statistically significant correlations for several classes of serum lipoproteins ($r = 0.28$ for the S_f 35 to 100 class), while the coefficient of correlation with total serum cholesterol was only $r = 0.10$. The values are based on 241 individuals and the difference between the two coefficients is significant statistically at the 5 per cent level. Biologically, the existing correlation is very low and accounts for only about 8 per cent of the *variance of the serum lipoproteins.* Nevertheless, the relationship is considered to have a

bearing upon atherogenicity. It is incorrect, however, to conclude that "obesity accounts for approximately 8 per cent of the relationship of the S_f 35–100 lipoproteins with *atherosclerosis*." The quantitative examination of this problem would require information on the correlation of each of the factors (obesity and the lipoproteins) with atherosclerosis and the calculation of coefficients of partial correlation.

Data on overweight as a contributing factor in the development of hypertension were reviewed by J. T. Sheridan and J. M. Peck (*Tr. Assn. Life Insurance Medical Directors of America* **35,** *291 (1952)*). Their sample of 231 employees who were followed for five years or more was subdivided into persons of light and medium weight (below 2.4 pounds per inch of height; number = 163), and of heavy weight (over 2.4 pounds; number = 68). Diastolic blood pressure exceeding 90 mm. of mercury, observed on one or more occasions, was over eight times as common in the heavy weight group. Of 59 persons with an intermittent or constant hypertension (blood pressure exceeding 140 systolic or 90 diastolic, or both), 66 per cent fell in the "overweight" class. Among individuals who were free of high blood pressure only 28 per cent were heavy.

These results are very similar to those obtained by K. J. Thomson (*Proc. 38th Annual Meeting, Medical Section, American Life Convention (June), p. 85 (1950)*) on the basis of annual health examinations of over 3000 individuals employed by the Metropolitan Life Insurance Company, extending over twelve years. At ages 35 to 44 the diastolic hypertension was almost three times as common, and at age 45 over twice as common among individuals of heavy weight as among those of light weight (less than 2.0 pounds per inch of height).

While much remains to be done to elucidate the etiology, the association between a variety of "degenerative" diseases and overweight is well established. Years ago this relationship was brought out in E. P. Joslin's (*Boston Med. Surg. J.* **186,** *833 (1922)*) comment that scales are to chronic disease what the thermometer is to acute illness.

THE RENAL CLEARANCE OF PANTOTHENIC ACID

Little is known about the mechanism of the renal excretion of the vitamins. There is considerable published work concerning the excretion of ascorbic acid. In the dog and man, at least, ascorbic acid is reabsorbed by the kidney tubule and a definite tubular maximum exists (H. W. Smith, "The Kidney, Structure and Function in Health and Disease," *Oxford University Press, New York (1951)*).

The renal handling of pantothenic acid is of particular interest. L. D. Wright *et al.* (*Am. J. Physiol.* **145,** *633 (1946)*) have studied pantothenic acid clearance in dogs. Under normal conditions much of the pantothenic acid of blood is protein-bound and little is available for filtration. With moderate pantothenic acid doses the clearance of pantothenic acid was not significantly different from the exogenous creatinine clearance. These results suggested that pantothenate was excreted by simple glomerular filtration without tubular reabsorption or excretion. This hypothesis has not been supported by more recent work. K. Roholt and V. Schmidt (*Scandinav. J. Clin. Lab. Invest.* **3,** *108 (1951)*) have investigated the renal excretion of pantothenate in man and found that at high levels of plasma pantothenate the clearance of the vitamin exceeded inulin clearance. At plasma pantothenate levels greater than 50 to 100 micrograms per milliliter there was a secondary fall in clearance which suggested that a tubular maximum had been exceeded. Strong support for the view

that tubular excretion occurred was obtained using carinamide (4′-carboxyphenylmethanesulfonanilide). This drug, which is known to inhibit the tubular excretion of penicillin, phenolsulfonphthalein and para-aminohippurate (PAH), was also found to inhibit the clearance of pantothenate.

Support for the hypothesis of tubular excretion of pantothenate has been published by W. P. Boger, G. M. Bayne, J. Gylfe, and L. D. Wright (*Proc. Soc. Exp. Biol. Med.* **82,** *604 (1953)*). These authors infused 500 and 1000 mg. of sodium pantothenate intravenously into 2 patients, respectively. The blood pantothenate was measured microbiologically with and without the preceding intravenous injection of 2.0 g. of probenecid (para-(di-n-propylsulfanyl)-benzoic acid). During the three hours following pantothenate loading, the pantothenate blood level fell more slowly when probenecid was given. In addition, the amount of pantothenate in the urine was less after the administration of the tubular blocking agent.

In another experiment, simultaneous determinations of the pantothenate, endogenous creatinine and PAH clearances were made in a patient. Following three control periods, 2.0 g. of probenecid were given intravenously and an additional gram was added to the sustaining solution which was infused slowly intravenously. During the control periods the pantothenate clearance was greater than the creatinine clearance, but was less than PAH clearance. When probenecid was given, pantothenate clearance fell to the same level as the creatinine clearance. PAH clearance fell also, but remained greater than endogenous creatinine clearance. Possible inhibition of pantothenate tubular excretion by PAH was sought. The clearances of pantothenate and endogenous creatinine were determined in the absence of PAH. The results were not altered by the omission of PAH. Probenecid again caused a fall of pantothenate clearance to the level of endogenous creatinine clearance.

From the evidence cited, it seems well established that at high plasma pantothenate levels there is an active process of tubular excretion, which can be blocked by carinamide and probenecid. The transport mechanism for pantothenate is probably shared with penicillin, PAH and phenol red, and other substances. As J. V. Taggart has pointed out in "Phosphorus Metabolism," (*vol. 2, p. 732, Johns Hopkins Press, Baltimore (1951)*), all compounds which share this excretory pathway are carboxylic acids with the exception of phenol red. The importance of phosphate bond energy and the possible role of coenzyme A, a pantothenic acid-containing molecule, in this transport has been suggested by Taggart.

As stated previously, the clearance of pantothenate is low at physiologic levels because of protein binding. Only when a high level of free pantothenate is maintained is it possible to demonstrate the tubular excretory mechanism. It is for this reason, as the authors comment, that no therapeutic implications of these studies are apparent.

BLOOD COPPER IN MAN

The metabolism of copper has received a good deal of attention in recent years, beginning with the important observation of a copper deficiency anemia (E. B. Hart, H. Steenbock, J. Waddell, and C. A. Elvehjem, *J. Biol. Chem.* **77,** *797 (1928)*). Important investigations have also revealed the effect of abnormalities in the diet, especially vitamin B_6 deficiency and a high phosphate diet, on copper absorption. It is known that copper is a co-factor for certain enzymes in the body. Finally, large accumu-

lations of copper have been found in the brain and certain other tissues in Wilson's disease. These and other aspects of copper metabolism have been discussed (*Nutrition Reviews* **4,** *227* (*1946*)).

A number of methods for the determination of copper in the blood have been developed. One of the most recent of these is that of C. J. Gubler *et al.* (*J. Biol. Chem.* **196,** *209* (*1952*)). Using this method, M. E. Lahey, Gubler, G. E. Cartwright, and M. M. Wintrobe (*J. Clin. Invest.* **32,** *322* (*1953*)) have studied the blood copper levels in normal human subjects and in patients with a number of disease states.

The normal subjects studied were medical students, doctors, nurses, or laboratory technicians between 20 and 40 years of age. They were stated to be "apparently healthy." The figures are first given for copper in the whole blood, in plasma, and in red blood cells in 40 male subjects and 23 females. These values in the females averaged somewhat higher than in the males. The whole blood copper for both sexes combined averaged 98 micrograms per 100 ml. with the standard deviation of 13. By calculation, using the red blood cell count, the volume of packed red blood cells and the red cell copper content, the mean corpuscular copper was found to be 100 micro-micro-micrograms. In a number of the subjects the diurnal variation in plasma copper was studied. In most instances there was a slight rise in the afternoon and a fall in the evening in the copper content, although there were a few notable exceptions. For 8 males blood and plasma copper were determined on successive days, but no significant variation was observed. Changes from week to week were likewise small and of questionable significance. There was but a slight rise in plasma copper due to food ingestion (two and one-half hours after the noon meal). Both the red blood cell and plasma copper values remained essentially unchanged during menstruation.

It was observed that the determined red blood cell copper was considerably higher than that calculated on the basis of the whole blood copper, plasma copper, and the volume of packed red blood cells. This was thought to be due to errors involved in each of the measurements required for the calculation, the most important being the whole blood copper.

In a subsequent report the same workers (M. E. Lahey, C. J. Gubler, G. E. Cartwright, and M. M. Wintrobe, *J. Clin. Invest.* **32,** *329* (*1953*)) describe the investigation of blood copper levels in pregnancy and certain diseases. The authors, in reviewing the previous study, note that, although a great deal of work had been done, considerable disagreement existed in many instances regarding the occurrence of abnormally high or low blood copper values in various disease states.

In pregnancy a definite and highly significant rise in the plasma copper was found during the last trimester. However, the red blood cell copper was normal, and the average corpuscular copper was 100 micro-micro-micrograms. As in pregnancy, so also in infections the red blood cell copper content did not change whereas the plasma copper and whole blood copper rose in most instances. Again, the mean corpuscular copper was normal. The mean value for plasma iron in the group with infections was low (50 micrograms per 100 ml.). The rate of removal of intravenously injected copper ("Cupralene") from the plasma was shown to be the same in a patient with chronic pulmonary tuberculosis as in the normal subject.

In acute leukemia most patients showed a high whole blood and plasma copper content, but an unchanged red blood cell copper value. A decrease in blood copper content was observed during improvement after the administration of ACTH to 2 patients with leukemia, with a reversal again when relapse occurred. In hemochromatosis not only were the plasma iron values usually elevated as expected, but

plasma copper was also increased. Pernicious anemia in relapse showed a tendency to a slight rise in both whole blood and plasma copper, but in many of the patients one or both determinations were normal. The mean cell copper was somewhat elevated (126 micro-micro-micrograms). No changes were observed in the blood copper content during the response to therapy in pernicious anemia, although hypoferremia was regularly observed during the period of rapid blood regeneration. Patients with iron deficiency anemia sometimes showed an elevation in the plasma copper content, which was most marked in infants. Hypocupremia was not noted in any case of iron deficiency anemia, nor was the mean cell copper in the infants with hypochromic anemia reduced (91 micro-micro-micrograms). The plasma iron concentration was diminished in all of the patients. It was emphasized by the authors that although copper deficiency may produce an anemia in infants, hypocupremia was not observed in the patients they studied.

There was an increase in the plasma copper level in aplastic anemia, but no change in the copper content of the red blood cells. There was an elevated plasma copper in hyperthyroidism, but it could not be correlated with the basal metabolic rate. In Wilson's disease, the plasma copper content was always low, while the red cell copper was normal. Likewise, in the nephrotic syndrome ("lipoid" nephrosis) both whole blood and plasma copper were diminished. In both of these diseases an increase in the urinary excretion of copper has been observed (*Nutrition Reviews* **7,** *255 (1949)*).

The authors concluded that there was very little fluctuation in the red blood cell copper content, and the changes in whole blood copper were usually due to a rise or fall in plasma content. The hypercupremia observed seemed to be nonspecific in that it occurred in a number of unrelated conditions.

A third paper by the same authors (C. J. Gubler, M. E. Lahey, G. E. Cartwright, and M. M. Wintrobe, (*J. Clin. Invest.* **32,** *405 (1953)*) presents data with respect to the transport of copper in the blood. It has been known since the work of C. G. Holmberg and C.-B. Laurell (*Acta chem. Scandinav.* **1,** *944 (1947)*; **2,** *550 (1948)*) that copper in plasma is bound to an alpha globulin which has a blue color and has been called ceruloplasmin, and is not bound to the $beta_1$ metal-binding globulin to which iron is attached. Another difference of ceruloplasmin from the iron-binding protein is that the latter under normal circumstances will bind additional iron *in vitro*, whereas ceruloplasmin appears to be saturated with copper. Because the ceruloplasmin seems at all times to be saturated and because there is little fluctuation of plasma copper following meals, the authors hypothesized that ceruloplasmin is not a means of active transport of copper.

Observations were made on man, the rat, dog and pig. Two copper fractions were found in the plasma—one which reacted directly with the reagent (sodium diethyldithiocarbamate), and the other which reacted only after treatment with acid, presumably the ceruloplasmin-bound copper. In man, approximately 96 per cent of the copper was found to be bound as ceruloplasmin, in the rat 99 per cent, in the dog 88 per cent, and in the pig 58 per cent. When copper was added to human, dog and pig plasma *in vitro*, not only could all of the copper be found in the direct-reacting fraction, but some additional copper seemed to have been liberated from the protein. In copper-deficient rats in which the plasma copper had been markedly reduced, from 90 to 100 per cent of the copper added *in vitro* reacted directly with the reagent, indicating no binding capacity even though the plasma copper was very low.

Fifty mg. of copper were given by mouth to 3 fasting human subjects (as Cupralene). There was no change in plasma or erythrocyte copper values during the eight hours of study. When a lethal amount of copper was given orally to a dog, there was a

striking rise in plasma copper, all direct-reacting, but an even more dramatic rise in the red cell copper.

Small amounts of copper given intravenously to 3 human subjects and one dog led to 90 per cent (human) to 100 per cent (dog) of the excess plasma copper reacting directly. This was cleared from the plasma in about four hours. There was some increase in the red blood cell copper in both man and the dog.

The authors concluded that ceruloplasmin probably does not transport copper in the same sense that the $beta_1$ iron-binding protein transports iron. It was thought more likely that copper transport is related to the direct-reacting plasma fraction, although it was thought possible that the red blood cells might be concerned under some circumstances. Finally, it was observed that the hypercupremia of pregnancy and infection was due to an increase in the indirect-reacting fraction; that is, the ceruloplasmin-bound copper.

FRUCTOSE AND INVERT SUGAR VERSUS GLUCOSE

Prior to the recent commercial development of fructose and invert sugar solutions for intravenous administration to patients, the potential nutritional advantages of hexose sugars other than glucose were largely of academic interest. Now that three different and, in a sense, competitive parenteral carbohydrate preparations are available to the physician, he has been forced to inquire into the relative merits of glucose, fructose and invert sugar (a mixture of equal parts of glucose and fructose) in the nutritional management of a variety of clinical problems.

Interest in fructose and invert sugar preparations has centered principally upon their potential usefulness in two important clinical situations; first, in the treatment of patients with impaired utilization of glucose, notably those with diabetes mellitus, and second, in the alimentation of patients to whom large quantities of calorigenic material must be given by the intravenous route.

Because fructose is phosphorylated through a hexokinase system distinct from that involved in glucose phosphorylation, utilization of it appears to be largely independent of influence from hormones which ordinarily regulate the rate of passage of glucose into cells (H. J. Deuel, *Physiol. Rev.* **16,** *173* (*1936*)).

M. Miller and his associates (*J. Clin. Invest.* **31,** *115* (*1952*)) studied the utilization of intravenously given fructose in patients with diabetes mellitus. They found that the fructose was well utilized by diabetic subjects as judged by fructose tolerance curves, changes in serum inorganic phosphate levels, and rises in blood levels of carbohydrate intermediaries such as pyruvate following fructose injection. Also, S. S. Chernick and I. L. Chaikoff (*J. Biol. Chem.* **188,** *389* (*1951*)) found that the livers from alloxan-diabetic rats were able to oxidize C^{14}-labeled fructose at a normal rate while conversion of C^{14}-labeled glucose to $C^{14}O_2$ under similar conditions was greatly inhibited.

Further detailed studies on the administration of fructose solutions to patients with impaired glucose tolerance due to diabetes mellitus or parenchymal hepatic disease have been reported by L. H. Smith, R. H. Ettinger, and D. Seligson (*J. Clin. Invest.* **32,** *273* (*1953*)). These authors found that the rate of disappearance of fructose from the blood has the characteristics of a "first order" reaction and that its rate of utilization appears to be directly proportional to its concentration in the blood. Although the fructose disappearance rates were measurably prolonged in patients with acute hepatitis, Laennec's cirrhosis and diabetes, impairment of fructose tolerance in such patients was relatively small and

not at all comparable to impairment in tolerance of the same patients to glucose.

When responses of serum inorganic phosphate to glucose and fructose respectively were compared it was noted that the phosphate levels fell more rapidly after fructose administration and also returned more rapidly to normal. Much higher levels of such carbohydrate intermediaries as pyruvate, lactate and alpha-ketoglutarate were found in the blood following fructose administration than could be measured after glucose administration. For this reason the authors suggested that a *qualitative* difference in the metabolic pathways of fructose and glucose may exist. Their suggestion was based partly on their conviction that fructose and glucose are metabolized at approximately the same rate.

Smith *et al.* (*loc. cit.*) also found that among nondiabetic subjects there was a slightly greater urinary loss of fructose than glucose when tolerance to the two solutions was successively measured in the same individual. The rates of administration employed were uniformly 0.5 g. of hexose per kilogram per hour.

Since glucose utilization is temporarily inhibited following a prolonged fast, attempts have been made to determine whether utilization of fructose would be similarly impaired under conditions of starvation. G. H. Wyshak and I. L. Chaikoff (*J. Biol. Chem.* **200,** *851* (*1953*)) have compared the relative abilities of liver slices from normal and fasted rats to metabolize radioactive glucose and fructose. It was learned that the ability of livers from rats fasted seventy-two hours to convert glucose to carbon dioxide decreased by about 50 per cent; however, livers from rats fasted under similar conditions showed no significant decrease in ability to oxidize fructose. Despite the difference in oxidation of the two sugars, it was of interest that virtually no lipogenesis from either of the added substrates took place at the end of the seventy-two-hour fast. Hepatic glycogenesis from C^{14}-glucose was markedly impaired by a forty-eight-hour fast. However, it was learned that conversion of C^{14}-fructose to glycogen was about the same in control and fasted livers.

In any comparative appraisal of glucose, fructose and invert sugar solutions, the problem of wastage of these preparations via the kidney must be considered. It is well known that when glucose solutions are administered more rapidly than 0.6 to 0.85 g. per kilogram per hour, significant amounts of the infused sugar are likely to be lost in the urine. Moreover, an associated diuresis involving undue excretion of water and electrolytes may occur at a time when this is particularly undesirable.

The reports of Miller *et al.* (*loc. cit.*) and Smith *et al.* (*loc. cit.*) indicate that glucose and fructose are roughly comparable from the standpoint of urinary loss. This is noteworthy in view of studies which indicate that the renal threshold to fructose is extremely low (Deuel, *loc. cit.*). Invert sugar solutions have been reported to be more readily retained in the body than either fructose or glucose (J. J. Weinstein, *Med. Ann. Dist. Columbia* **19,** *179* (*1950*)). However, later studies by J. L. Smith, J. M. Beal, and P. Frost (*Surgery* **31,** *720* (*1952*)) have failed to disclose any advantage of invert sugar over glucose with regard to "renal tolerance." If mixtures of glucose and fructose eventually are shown to be somewhat better retained in the body than either glucose or fructose alone, the explanation presumably will be that the kidney is handling the simultaneously administered hexoses as separate rather than additive substances (J. P. Peters and D. D. Van Slyke, "Quantitative Clinical Chemistry," *Vol. 1, 2nd edition, p. 133, Williams and Wilkins, Baltimore* (*1946*)).

On the basis of work published to date, it appears that fructose ultimately may prove to be the sugar of choice in the parenteral nutrition of certain patients in whom glucose utilization is impaired. Dia-

betes mellitus and severe parenchymal liver disease are examples of conditions in which fructose administration sometimes may be preferable. "Starvation diabetes" is ordinarily a transient problem and it is debatable whether in such instances fructose is to be preferred over glucose. There is some experimental and theoretic evidence (*loc. cit.*) to support the view that invert sugar may be given with less urinary loss of the carbohydrate and hence more rapidly than either glucose or fructose alone.

However, in view of conflicting reports about renal tolerance to invert sugar and fructose (J. Bertino *et al.*, *J. Clin. Endocrinol.* **13,** *658 (1953)*; J. M. Beal, J. L. Smith, and P. M. Frost, *Surgery* **33,** *721 (1953)*), further studies to resolve this question are needed. For example, Beal *et al.*, while comparing tolerance to glucose and fructose solutions respectively, collected urine samples for fructose and glucose over a longer period than did Bertino *et al.*, T. E. Weichselbaum, R. Elman, and R. H. Lund (*Proc. Soc. Exp. Biol. Med.* **75,** *816 (1950)*), or J. J. Weinstein (*J. Lab. Clin. Med.* **38,** *70 (1951)*). Beal and his associates found that significant quantities of reducing sugars often were still present in the urine four and five hours after completion of the injection. These differences in duration of collection period may account for some of the discrepancies found in the various published reports on the subject. Rates of administration of the various sugar preparations have varied widely from study to study; this also has made interpretation of the results difficult. Lastly, it should be borne in mind that even if invert sugar (or fructose) should prove to be retained in the body to a slightly greater extent than is glucose, the advantages from the standpoint of caloric intake will be negligible (*Nutrition Reviews* **9,** *173 (1951)*).

Clinical and experimental knowledge about fructose as a parenteral nutrient still is limited. Studies should be done to determine whether respiratory or metabolic acidosis is significantly aggravated by the increased production of organic acids which accompanies fructose administration. On theoretic grounds, it is difficult to imagine that fructose administration would be harmful in such conditions since the amount of lactic acid produced when fructose is given is relatively small (L. H. Smith *et al.*, *loc. cit.*). Cerebral metabolism of fructose is not well understood and, indeed, certain details of its intermediary metabolism appear to require elucidation. When these questions have been satisfactorily answered it may be predicted that fructose-containing preparations will find a secure place on the roster of useful parenteral aliments.

LEAN BODY MASS AS A METABOLIC REFERENCE STANDARD

In studies of energy metabolism three criteria have been used as expressions of "body size" (*Nutrition Reviews* **10,** *42 (1952)*): (1) gross body weight (W), (2) body weight raised to a power (W^n, where n is frequently taken as 0.73; (*see* "Calorie Requirements," *FAO Nutritional Studies No. 5, Washington (1950)*), and (3) body surface estimated from height and weight. It is the latter value which has become a preferred reference standard, and the evaluation of an individual's basal metabolism is carried out routinely by referring to the oxygen consumption (per unit of time) per square meter of body surface.

The customary calculation of basal energy expenditure in terms of body surface has the undeniable virtue that it reduces the large interindividual variability present when oxygen consumption is expressed in absolute terms, *i.e.*, as oxygen consumption, in cubic centimeters per individual. The per m^2 expression is useful for evaluating the degrees of departure, in disease, from

the norm. However, there is adequate evidence that the surface area of the body does not directly *determine* the basal energy expenditure. Consequently, expressing the oxygen consumption per unit of surface area does not have a special, intrinsic, theoretic merit. It does not increase our understanding of the phenomena involved. In order to describe the calorie expenditure more adequately, it is desirable to relate the basal energy exchanges to a unit mass of the metabolizing tissue. Thus we obtain a direct measure of the intensity of the metabolic process (A. Keys *et al.*, "The Biology of Human Starvation," *p. 337, Univ. of Minnesota Press, Minneapolis (1950)*).

Gross body weight is an unsatisfactory reference point because of the large variations in body composition in individuals of the same body weight. In a normally hydrated body, the fat depots constitute a segment of body weight characterized by a low energy metabolism. Consequently, the separation of body weight into its "fat" and "fat-free" compartments is the first and important step in the search for a proper measure of the "metabolic body size," useful as a reference standard in studies of energy metabolism, nutritional requirements and drug dosage.

A. R. Behnke (*Fed. Proc.* **11,** *11 (1952)*) noted that "lean body mass," defined as body weight less "excess (or nonessential) body fat" and determined from specific gravity and body water determinations, can be estimated with fair accuracy from basal metabolism data. However, Behnke's own data counsel caution. Thus, in 129 Navy men the coefficient of correlation between basal metabolism and lean body weight was only moderately high (r = 0.799), leaving 36 per cent of the variance unaccounted for (Behnke, *Tr. N. Y. Acad. Sci. Ser. II*, **15,** *74 (1953)*).

Of interest is the high correlation between surface area (D. DuBois and E. F. DuBois, *Arch. Int. Med.* **17,** *863 (1916)*) and the lean body mass estimated densitometrically (r = 0.941). However, it should be noted that the equations for estimation of lean body mass from surface area depend on the assumed fat content of the body. Behnke gives "representative" values for young (24 to 25 years) and middle-aged (55 years) men as 10 and 19 per cent. For young women an estimated value of 15 per cent is given. All of these values are lower than the empiric values obtained by J. Brozek, K.-P. Chen, W. Carlson, and F. Bronczyk (*Fed. Proc.* **12,** *21 (1953)*). By use of a linear fit, men averaging 25, 35, 45 and 55 years were shown to have 13.1, 17.3, 21.6 and 25.9 per cent of body weight as fat. Women exhibited a similar age trend, but the values for the four age groups were uniformly higher (26.5, 30.5, 34.5 and 38.5 per cent). In studies on aging and on sex comparisons the sampling plays a crucial role. Ideally, only samples of standard weight for height or of a given degree of underweight or overweight should be compared.

The relationship between lean body mass and basal metabolism was also examined by A. T. Miller and C. S. Blyth (*J. Applied Physiol.* **5,** *73 (1952)*). Again, the lean body mass was calculated by subtracting densitometrically determined body fat from total body weight. In their series the correlation with basal oxygen consumption was high (r = 0.924), allowing a satisfactory estimate of the lean body mass from basal oxygen consumption. The accuracy of the prediction equation refers to the given experimental procedures, involving triplicate determinations of the basal metabolic rate as a minimum.

It should be kept in mind that the observed correlation cannot be regarded as conclusive proof of the validity (absolute accuracy) of the methods used for arriving at the particular values of "lean body mass." The fact is that the two series of values (oxygen consumption and "lean body mass") vary fairly closely together. A systematic

error in one variable, that is, a constant fraction added to or subtracted from the calculated values of "lean body mass," would not affect the degree of correlation. This is due to the fact that in calculating a coefficient of correlation one considers the *deviations* from the means of the two variables, not the absolute values.

Miller and Blyth examined also the correlation of lean body mass with creatinine excretion. The association was less close (r = 0.826) and the error of estimation proportionately larger. The correlation between relative body weight and fat was too low to be useful for predictive purposes. It was emphasized that both the oxygen consumption and creatinine excretion may be influenced by a variety of factors unrelated to real differences in lean body mass and that the conditions of testing must be carefully standardized if the prediction equations are to be applicable. When the prerequisites are satisfied, the approach should be useful in circumstances where more direct measurements of body fat would be inconvenient or impossible.

W. R. Best, W. J. Kuhl, and C. F. Consolazio (*Fed. Proc.* **12,** *178 (1953)*) calculated coefficients of correlations between oxygen consumption and creatinine excretions, and various estimates or indicators of lean body mass based on direct physical measurements (height, weight, circumferences, skinfolds) and roentgenograms of the right upper arm used for the calculation of lean brachial mass and brachial muscle mass. The highest coefficients of correlation were obtained for lean body mass obtained by estimating body fat on the basis of skinfold measurements (r = 0.885 for oxygen consumption, r = 0.610 for creatinine excretion).

In a subsequent paper by A. T. Miller and C. S. Blyth (*J. Applied Physiol.* **5,** *311 (1953)*) the argument is centered on the use of the lean body mass as a suitable metabolic reference standard. It is argued that the addition of fat, which is nearly inert as far as energetic exchanges are concerned, should influence total oxygen consumption less than the addition of an equal mass of "active tissue," for example, muscle, although the increase in the calculated surface area would be identical. Ideally, then, one should calculate in terms of the "active tissue mass" (Behnke's active protoplasmic mass). This component is not susceptible of direct measurement. Both Behnke, and Miller and Blyth assume that the "lean body mass" is relatively constant in composition and that, in the adult, it incorporates as a constant fraction the active tissue mass. It is the fat content which represents the most variable component of body weight, and age and sex differences in the basal metabolism of adults can be accounted for mainly on the basis of variation in the "dispensable" (excess) fat content (Behnke, *loc. cit.*).

The thesis examined by Miller and Blyth states that the lean body mass is a better reference standard than either body weight or the surface area, both of which are influenced by the variable, energetically nearly inert fat. The data provide an inconclusive support of their thesis. The coefficient of correlation between basal oxygen consumption and lean body mass (r = 0.92) was somewhat higher than for surface area (r = 0.84) and body weight (r = 0.85). However, the differences do not reach the 5 per cent level of statistical significance.

This does not mean that the concept is useless and the search for a better standard of reference futile. In predicting basal oxygen consumption from a combination of the three factors (body weight, surface area and lean body mass), the lean body mass is the best single predictor. Due to the intercorrelations among the three predictors, the accuracy of prediction increases very little when body weight and surface area are added. More important, perhaps, than these purely statistical exercises are empiric data on conditions in which large changes

in the basal oxygen consumption were observed.

It has been pointed out by Keys *et al.* (*loc. cit.*) that during a prolonged period of drastic caloric reduction and subsequent refeeding the *direction* of the changes in basal metabolism was the same, irrespective of the units in terms of which it was expressed. However, *quantitatively*, the results obtained by using the various methods of calculation—per individual, per kilogram of body weight, per square meter of body surface, per kilogram of "active tissue"—were not identical. As to the interpretation of the results, important points might be decided in different ways according to the method used for expressing the raw data on oxygen consumption.

During semistarvation and subsequent nutritional rehabilitation the basal metabolism appeared to vary least when expressed per kilogram of "active tissue," where the latter value represented the residue of body mass not accounted for by body fat, extracellular fluid, and bone mineral. Similar experience has been encountered in a study on the effects of aging (J. Brozek, *Fed. Proc.* **11,** *784 (1952)*).

THE EFFECT OF VITAMIN B_{12} ON SULFHYDRYL COMPOUNDS

Evidence suggesting an influence of vitamin B_{12} on methionine metabolism was presented several years ago (*see Nutrition Reviews* **8,** *301 (1950)*). Before vitamin B_{12} was isolated in pure form it was observed that deficiencies of the "animal protein factor" were produced most readily when the animals were fed a diet deficient in methionine. Subsequent work using pure preparations of vitamin B_{12} has supported these observations. It was shown by J. S. Dinning, L. D. Payne, and P. L. Day (*Arch. Biochem.* **27,** *467 (1950)*) that leukopenia developed when rats were fed a purified diet deficient in methionine and vitamin B_{12}. This leukopenia could be prevented by feeding methionine alone, or by feeding betaine only when vitamin B_{12} was added to the diet. These results suggested that vitamin B_{12} was necessary for the utilization of betaine for methionine synthesis. T. H. Jukes, E. L. R. Stokstad, and H. P. Broquist (*Ibid.* **25,** *453 (1950)*) were able to demonstrate that vitamin B_{12} was necessary for the utilization of homocystine by chicks fed a methionine-deficient diet.

E. L. Oginsky (*Arch. Biochem.* **26,** *327 (1950)*) studied the formation of methionine *in vitro* by liver homogenates from normal and vitamin B_{12}-deficient rats. It was found that vitamin B_{12} deficiency greatly reduced the ability of liver homogenates to methylate homocysteine.

J. W. Dubnoff (*Arch. Biochem.* **27,** *466 (1950)*) reported that the *in vitro* addition of vitamin B_{12} preparations to incubation mixtures of homocysteine, betaine and rat liver increased the amount of methionine formed. In other experiments a study was made on the effect of vitamin B_{12} added *in vitro* on the oxidation of sulfhydryl groups. Homocysteine or homocystine was incubated with rat liver slices and the amount of sulfhydryl was measured. It was found that vitamin B_{12} reduced the oxidation of the –SH group and increased the reduction of the S—S group. Similar results were obtained with oxidized and reduced glutathione. These observations suggested that vitamin B_{12} enhanced the formation of methionine by reducing the oxidation of the –SH group of homocysteine which is the methyl acceptor. Since many enzyme systems require intact –SH groups for activity this action of vitamin B_{12} could produce many metabolic effects.

In view of the above considerations the report of C. T. Ling and B. F. Chow (*J. Biol. Chem.* **202,** *445 (1953)*) is of considerable interest. A study was made of the

effect of vitamin B_{12} on the level of soluble sulfhydryl compounds in red blood cells. Experiments were conducted using vitamin B_{12}-deficient rats, folacin-deficient rats, iron-deficient rats, and patients with pernicious anemia. Blood was taken from rats by cardiac puncture and from pernicious anemia patients by venipuncture. The blood was centrifuged, the plasma was discarded and the red cells were resuspended in saline. The cells were hemolyzed, the protein was precipitated by sulfosalicylic acid, and total sulfhydryl compounds were determined in the protein-free filtrate. The results are reported as micromoles of sulfhydryl groups per 100 ml. of blood cells. In other experiments glutathione and ergothioneine were determined directly. It was found that by this method all the total sulfhydryl compounds of red blood cells could be accounted for by glutathione.

In the experiments with vitamin B_{12}-deficient rats, two diets were employed. One diet consisted of 60 per cent soybean meal and 40 per cent sucrose with the usual vitamins and was fed to rats 35 to 40 days of age at the start of the experiment. The other diet contained the following components, in per cent: soybean meal, 68; cottonseed oil, 4; sucrose, 24; salts, 4; and the usual vitamins, and was fed to adult rats. The rats were fed these diets for from three to seven weeks. Controls were rats receiving the deficient diet and injected with 25 micrograms of vitamin B_{12} subcutaneously six times per week. In the first series of young rats after a five-week feeding period the final average body weights were: vitamin B_{12}-deficient, 59 g.; deficient injected with vitamin B_{12}, 138 g.; normal, 191 g. The average –SH content of erythrocytes for the above three groups were: 75, 318, and 248 micromoles per 100 ml. respectively. In the second series which consisted of adult rats, after a seven-week feeding period the average –SH content of the red blood cells were: vitamin B_{12}-deficient, 123; deficient injected with vitamin B_{12}, 206; normal, 194 micromoles per 100 ml. In the third series in which young rats were fed the diet for three weeks the –SH values were: deficient, 129; deficient injected with cobalt chloride, 117; normal, 222 micromoles per 100 ml. The differences observed in blood sulfhydryl content between vitamin B_{12}-deficient and vitamin B_{12}-injected rats were statistically significant. These results show that vitamin B_{12} deficiency in the rat results in a lowered sulfhydryl content of the red blood cells. This reduction is prevented by injection of vitamin B_{12} but not by cobalt chloride.

In order to determine if other disturbances in hematopoietic functions would produce similar reduction in –SH content, Ling and Chow studied folacin deficiency and iron deficiency anemia in rats. In the folacin experiments young rats were given a purified diet containing sulfasuxidine until they developed diarrhea and leukopenia. Controls were injected with folacin. For the production of iron deficiency anemia weanling rats were placed in enamel cages and given a diet consisting of whole milk powder, iron-free salts and a vitamin supplement. Controls were stock rats. The results of determinations of red cell –SH groups were: folacin-deficient, 251; folacin-treated, 248; iron-deficient, 192; normal, 200 micromoles per cent. Thus neither folacin nor iron deficiency affected the content of –SH groups in the red cells. These results then indicate that the vitamin B_{12} effect is specific and is not simply a reflection of impaired hematopoiesis.

Vitamin B_{12} is considered the specific treatment for pernicious anemia. It was therefore of interest to determine the effect of pernicious anemia and therapy with vitamin B_{12} on the concentration of –SH groups in the red cells. Four patients with pernicious anemia in relapse were studied. Blood was drawn initially and then at intervals after vitamin B_{12} therapy. In all cases the injection of 3 mg. of vitamin B_{12} resulted in a considerable increase in the concentration

of sulfhydryl compounds in the red cells. Data were not given for the sulfhydryl content of red cells from normal persons, but it seems reasonable to assume that the increase in response to vitamin B_{12} injections in pernicious anemia patients indicates that when these patients were in relapse the red cell sulfhydryl content was below normal.

The results of these experiments provide additional evidence to indicate that a major metabolic effect of vitamin B_{12} is to maintain sulfhydryl groups in the reduced form. Since sulfhydryl groups occur in enzymes which function in all stages of growth and development the many diverse effects of vitamin B_{12} deficiency could be explained on this basis.

FUNCTIONAL VASCULAR DEFECTS IN EXPERIMENTAL SCURVY

The morphologic alterations of blood vessels in scurvy are well recognized, but the functional vascular changes are less well understood. An experimental approach to this problem has been made by R. E. Lee and N. Z. Lee (*Am. J. Physiol.* **149,** *465 (1947)*), who have reported observations on the mesenteric vessels of unanesthetized scorbutic guinea pigs. A decreased contraction of vessels was observed following the topical application of physiologic concentrations of epinephrine to the mesentery. This change was most marked in the arterial portion of capillaries beyond the pulsatile arteries. The terminal collecting venules tended also to become dilated, engorged, and to be easily ruptured by trauma.

The ability of scorbutic guinea pigs to withstand hemorrhage has been found to be impaired (R. E. Lee and E. A. Holze, *Proc. Soc. Exp. Biol. Med.* **76,** *325 (1951)*). The susceptibility to hemorrhage in scurvy may be related to vasohumoral agents, because the plasma from scorbutic guinea pigs subjected to hemorrhage does not contain vasoexcitatory material (VEM) when tested on the rat's mesoappendix by the method of Chambers and Zweifach. The plasma of control guinea pigs invariably contains VEM after hemorrhage.

Direct evidence that the absence of VEM in scorbutic guinea pigs can be attributed to impaired renal production has been reported (R. P. Akers and R. E. Lee, *Proc. Soc. Exp. Biol. Med.* **82,** *195 (1953)*). The liberation of VEM into an incubation medium by kidney slices from normal and scorbutic guinea pigs was measured. A control series of guinea pigs was pair-fed the scorbutogenic diet and also received 20 mg. per day of ascorbic acid.

When the kidney slices were incubated in a nitrogen atmosphere the slices from control guinea pigs in all cases elaborated VEM. When guinea pigs had been on the low ascorbic acid diet for seven days kidney slices from 2 of 5 guinea pigs failed to elaborate VEM. After fourteen and twenty-one days of the experimental diet none of the kidneys from scorbutic guinea pigs produced VEM. Incubation of the kidney slices in oxygen instead of nitrogen prevented the elaboration of VEM by both control and ascorbic acid-deficient guinea pigs. It is reasonable to assume that the VEM was really liberated in the oxygen control series because the initial Ringer-phosphate buffer wash before incubation of the tissue was devoid of VEM.

The interpretation of these results in terms of the disturbance in scurvy is difficult. VEM is measured by sensitization of small vessels of the rat mesoappendix to topical epinephrine. However, when epinephrine is given by intravenous or intra-arterial injection a normal pressor response occurs in scorbutic guinea pigs (Lee and Holze, *loc. cit.*). The studies reviewed do indicate that functional as well as structural vascular defects may be demonstrated in experimental scurvy.

MODIFICATION OF THE EFFECTS OF CHOLESTEROL FEEDING IN THE CHICK

Atherosclerosis has been observed to occur spontaneously and commonly in chickens, with many similarities to the disease in man. The susceptibility of chickens to atherosclerosis has been further demonstrated by the experimental production of the lesion at an early age by inclusion of added quantities of cholesterol in the feed (*Nutrition Reviews* **9,** *338 (1951)*). Evidence has been presented indicating that the production of atherosclerosis and the hypercholesterolemia associated with it are independent of the caloric content of the diet, but seem to be related more importantly to the quantity of cholesterol ingested (S. Rodbard, C. Bolene, and L. N. Katz, *Circulation* **4,** *43 (1951)*). Little information is available concerning other factors in the diet or patterns of feeding as they relate to the production of experimental atherosclerosis in the chick.

The results of experiments designed to determine the effect of fasting or the intermittent feeding of diets supplemented with cholesterol have been published recently (S. Rodbard, C. Bolene-Williams, R. Pick, and L. N. Katz, *J. Lab. Clin. Med.* **41,** *587 (1953)*). The chicks used were newly hatched cockerels. In a first experiment groups of chicks were fed a diet containing added cholesterol on alternate days. The birds were killed at intervals up to twenty-six weeks and the occurrence of atheroma in the thoracic aorta was determined. Blood specimens for determination of plasma cholesterol were obtained at selected intervals throughout the course of the experiment.

Atherosclerosis was not found in the control animals fed chick mash. Lesions of moderate severity occurred in 12 of 16 chicks provided daily with mash containing 2 per cent cholesterol. Other animals given the cholesterol-supplemented mash and fasted on alternate days had an even greater incidence of atheroma which were of higher average grade. All of the 14 chicks in this group were affected. The provision of normal mash without added cholesterol rather than fasting on alternate days caused an amelioration of the effect of cholesterol feeding. Ten of 21 chicks in a group so handled had atheroma in early and less severe form.

Correspondingly it was found that plasma cholesterol concentrations were increased markedly with the inclusion of cholesterol in the diet. However, values were even higher in the animals fed the high cholesterol diet and fasted on alternate days. This occurred despite the fact that these chicks received only slightly more than half the food consumed by those in the other groups. The apparent ameliorative effect of alternately feeding normal mash was exhibited in plasma cholesterol determinations, the average concentration at the end of the twenty-six-week period being less than half that determined in the group of chicks which were fasted on alternate days.

Another group of experiments was undertaken in which the period of fasting or intermittent feeding was extended to five days. The beneficial effect of feeding normal mash in the alternate period was again demonstrated. The diets of these animals were supplemented with 5 per cent cottonseed oil. Consistent feeding of cholesterol with the added oil produced a great elevation in plasma cholesterol; mean concentrations of 2017 mg. per cent were recorded after fifteen weeks on the diet. This compared with 102 mg. per cent in control chicks fed mash. When animals were fed this diet for five days and then fasted for five days, the elevation in plasma cholesterol was not so great, and decreased nearly 50 per cent in the fasting period. Normal mash fed in similar intermittent periods modified the influence on plasma cholesterol markedly. Increase of the oil content from 5 to 20 per cent in the diet of normal mash, fed in the alternate period, was associated with a somewhat greater elevation of cholesterol in plasma.

These observations provide evidence that the constancy of cholesterol intake impor-

tantly conditions the occurrence of hypercholesterolemia and atherosclerosis in chicks. This factor is apparently of greater importance than the quantity of cholesterol fed, since chicks consuming only half as much food and cholesterol but intermittently fasted had greater severity of lesions and higher levels of plasma cholesterol than those fed the high cholesterol diet ad libitum. Moreover, it is demonstrated that factors in the normal diet protect against this defect, since there was a rapid decrease in cholesterolemia and in severity of atheromatous lesions when animals were alternately fed normal mash. Larger quantities of fat in the diet prevented the protective effect of normal mash. These findings may indicate that the gastrointestinal tract effects important controls on the absorption and elimination of cholesterol.

The finding that intermittent fasting enhances the effect of cholesterol feeding on atherosclerosis should be viewed in light of other experimental observations. It has been repeatedly observed that fasting and inanition cause reduction in the level of plasma and tissue cholesterol in other species (*Nutrition Reviews* **10,** *345 (1952)*). Furthermore, fasting has been found to depress markedly the rate of endogenous synthesis of cholesterol from C^{14}-labeled acetate by the rat liver (G. M. Tomkins and I. L. Chaikoff, *J. Biol. Chem.* **196,** *569 (1952)*). The data of Rodbard and his associates (*loc. cit.*) demonstrate that the high level of plasma cholesterol occurring in cholesterol-fed chicks decreases during intermittent periods of fasting. Although it is warranted to conclude that cholesterol from endogenous sources as well as that from the diet is reduced during fasting, this apparently is not protective against further progression of the atheromatous process.

FAT DEFICIENCY AND ENDOCRINE FUNCTION

Although many investigators have studied the influence of low fat diets in the rat, the influence of such diets on the endocrine system has received little attention. In the early work following the discovery of the importance of fat in the diet, irregular ovulation and impaired reproduction were established as a part of the deficiency syndrome (G. O. Burr and M. M. Burr, *J. Biol. Chem.* **82,** *345 (1929)*; **86,** *587 (1930)*). T. C. Panos and J. C. Finerty (*J. Nutrition* **49,** *397 (1953)*) have recently reported a comprehensive study in which they have attempted to correlate certain of the structural tissue changes in fat-deficient animals with apparent disturbances in endocrine function.

One hundred female weanling rats were placed on an 18 per cent protein, low fat diet containing all other known dietary essentials. After twenty-four weeks, 30 of the animals were killed for study of the tissues while remaining animals were placed in four dietary groups: (1) fat-free diet; (2) fat-free diet plus 20 mg. of methyl linoleate per rat daily; (3) fat-free diet plus 40 mg. of methyl linoleate per rat daily; and (4) complete laboratory chow diet. These animals were killed for study after ten weeks on the respective diets. An additional 16 animals were fed the laboratory chow diet from weaning.

All animals developed the fat-deficiency dermatitis of the tail and feet. Complete reversal of these changes occurred among the animals transferred to the chow diet. The animals receiving the methyl linoleate supplement exhibited marked, but less complete, recovery. The time of vaginal opening was not affected by fat deficiency. Irregularity of estrus was a constant finding in 60 per cent of the animals after the twentieth week of the experiment. The irregularity consisted of variable prolongation of the cycle, frequently with runs of diestrus. This disorder was not corrected by methyl

linoleate. Estrus cycles were regular in all the animals receiving the chow diet.

When computed on the basis of organ weight per unit of total body weight, the thyroids and adrenals were smaller, while the kidney, heart and liver were larger in the fat-deficient animals than in the control animals. The enlargement of the kidneys was particularly pronounced; they were 43 per cent larger in fat-deficient animals that weighed 20 per cent less than the controls. In the fat-free animals, the outstanding microscopic finding in the kidneys was the large accumulation of blood between the tubules. There was also a variable amount of intratubular debris, and enlargement and congestion of the glomerular tufts. Some evidence of capillary hyperemia persisted in the animals given methyl linoleate or chow diet during the ten-week recovery period. Animals on the latter diet, however, had recovered to a nearly normal state.

Ovaries of the fat-free group contained a larger amount of interstitial tissue than those receiving fat. The most striking finding in the ovaries was a large number of "wheel nuclei," presumably evidence of a deficiency of pituitary secretion of the luteinizing hormone.

Fat deficiency resulted in a marked reduction of acidophil cells in the pituitary and an increase in the basophils. The marked fall in acidophils in this gland together with the presence of "wheel nuclei" in the ovaries and disturbed ovulation lead the authors to the contention that one of the important consequences of fat deficiency is a decrease in the secretion of luteinizing hormone by the pituitary gland. The increase in pituitary basophils was interpreted as evidence of a decrease in the secretion of estrogen by the ovaries. Absence of estrogen production is known to lead to an increase in pituitary basophilic cells.

The histologic findings in the ovary and pituitary in fat-deficient rats as described by these investigators seem to agree reasonably well with similar changes that have been obtained by ovary-pituitary imbalances in endocrinologic studies. This study deserves particular attention since all too often nutritional studies fail to recognize to what extent deficiency signs may actually represent disturbances of an endocrine type. The manner in which essential fatty acids or fat enter into ovarian and pituitary tissue metabolism still needs to be determined.

FACTORS INFLUENCING THE LEVEL OF FREE CHOLINE IN PLASMA

The metabolic fate of choline in the body has not been fully elucidated. Observations of H. Popper, J. de la Huerga, and D. Koch-Weser (*J. Lab. Clin. Med.* **39,** *725 (1952)*; *Nutrition Reviews* **10,** *278 (1952)*) demonstrate that trimethylamine is the chief urinary excretion product of choline given orally to rats or human subjects. Moreover, they found that the conversion of choline to trimethylamine occurs in the intestinal tract and is the result of bacterial action. When choline was administered intravenously no increase in urinary trimethylamine occurred.

Other aspects of choline metabolism have been investigated and are reported in a series of recently published papers. One approach was concerned with the determination of free choline in plasma of man and experimental animals under a variety of conditions. A biologic method for the assay of choline was utilized (J. Bligh, *J. Physiol.* **117,** *234 (1952)*). It was found that the quantity of free choline in plasma is relatively small and remarkably constant in human subjects. The range of values found was from 1.0 to 2.0 micrograms per milliliter, with a mean of 1.4 micrograms per milliliter in normal subjects. Similar levels were found in other species, *i.e.*, the dog, cat and rat.

In other experiments the same investi-

gator has attempted to determine the roles of the liver and kidney in controlling the quantity of circulating free choline (J. Bligh, *J. Physiol.* **120,** *53* (*1953*)). He found that intravenous injection of 50 or 100 mg. of choline chloride into dogs produced a marked elevation in the free choline concentration of plasma, followed by a rapid decrease within five minutes and a fall to a level comparable to that found prior to injection within twenty minutes. The sharp early fall was considered to be due to free diffusion into extracellular fluid, while the less rapid decrease occurring later in the postinjection period was thought to result from active removal of choline from the blood stream, perhaps through the activity of choline oxidase in tissues. About 10 per cent of the dose administered was recovered from the urine in the hour immediately following injection.

To assess the role of the kidney in this process of clearance of free choline from the plasma, similar experiments were done in dogs after ligation of the renal arteries and veins of both kidneys, or after bilateral nephrectomy. The rate curves of disappearance from the blood of the injected choline were similar to those in normal dogs, indicating that the kidney is not the organ primarily responsible for the rapid removal of the intravenously administered choline.

The liver was removed from the circulation of other dogs by simple evisceration and ligation of the vessels to this organ. In these animals at rest there was a small increase in the level of free choline in plasma, but only slight interference with the removal of injected choline. These observations seemed to indicate that the liver is not essential for the clearance of added choline, but may have a role in the finer control or maintenance of normal concentrations of plasma choline.

When the liver and both kidneys were removed, there was a marked effect on the rate of clearance of injected choline. The rapid fall in plasma choline resulting from diffusion into body tissues persisted, but the rate of removal thereafter was markedly diminished. Preinjection concentrations were not reached until sixty to one hundred and twenty minutes. This compared with twenty minutes in the earlier experiments with normal dogs, or after bilateral nephrectomy or removal of the liver. The author seems to have offered adequate evidence that the liver and kidneys, together, are importantly concerned in the clearance of added choline. However, other tissues may contribute to this function, but are apparently not primarily responsible or able to remove rapidly any significant quantity of injected choline.

The free choline of plasma was determined in 15 patients with hepatic disease and in 6 others with renal tubular dysfunction. No significant variation from normal levels was found, indicating that it is unlikely that diseases of these types are associated with an increase in plasma choline. It therefore becomes improbable that physiologic or pathologic events will lead to high levels of free choline in plasma, since the body possesses mechanisms to remove rapidly comparatively large quantities of added choline from blood.

The author turned his attention to the changes in plasma choline which might be associated with choline deficiency (J. Bligh, *J. Physiol.* **120,** *440* (*1953*)). In these experiments rats were placed on a choline-deficient diet at the age of 2 months and were sacrificed after sixteen weeks on the diet. Control groups of animals received a usual laboratory rat diet or the deficient diet plus added choline. The livers of the deficient rats were found to have a large accumulation of fat, the fat content averaging nearly 32 per cent as compared to values of about 7 per cent in the control groups. The deficiency was associated also with a decrease in the level of plasma choline, the average value being 0.8 micrograms per milliliter. This represented a reduction due to prolonged dietary deficiency of about 50 per cent of the normal level. Other in-

vestigators have demonstrated that choline may be derived by biosynthesis in the rat and that this process is not interfered with even in choline deficiency (C. H. Best and C. C. Lucas, *Vitamins and Hormones* **1,** *1* (*1943*)). The experiments of Bligh (*loc. cit.*) demonstrate quite definitely that this process is not sufficient to interfere with the production of fatty livers or reduced concentrations of plasma choline when diets deficient in choline are fed.

The observations described are worthy of note since they add significantly to the rather limited information concerning the fate of choline in the body. Pursued further they may contribute importantly to an understanding of the metabolic pathways taken by choline.

RELATIVE HUMIDITY AND NUTRITIONAL PATHOLOGY

The need for properly air-conditioned animal quarters in nutritional experimentation has been stressed in many symposiums on the care of laboratory animals. In part this has been based on the desire of the investigator to eliminate as many variables as possible from the experimental picture. In part it stems from the recognition of the influence of environmental temperature upon food intake and metabolism of the experimental animal. Until the publication of a series of papers from the University of Wisconsin there has been only little appreciation of the possible effect of relative humidity on the outcome of nutritional studies.

A paper by M. Schreiber, R. A. Collins, H. Niño-Herrera, and C. A. Elvehjem (*J. Nutrition* **48,** *125* (*1952*)) demonstrated the striking influence of relative humidity on the dermatosis developing in weanling rats fed lactose-containing diets. The feeding of mineralized milk or purified lactose-containing diets led to the development of dry, scaly dermatosis on the paws of weanling rats. Maximal severity of the lesion occurred after ten to twelve days on the experimental diet and was followed two to three weeks later by spontaneous recovery. (The time sequence here reported is somewhat reminiscent of that observed on diets mildly deficient in pantothenic acid, in which symptoms of deficiency occur during the animal's period of active growth and may disappear when the growth rate diminishes.) The lactose dermatosis could be prevented by maintaining the animals in an environment in which the relative humidity was maintained at about 50 per cent. Lowering the relative humidity progressively aggravated the severity of the lesions.

More recently, H. Niño-Herrera, M. Schreiber, R. A. Collins, and C. A. Elvehjem (*J. Nutrition* **49,** *99* (*1953*)) have described in more detail the histologic picture of the lactose dermatosis and the effect of relative humidity upon it. The weanling rats used in this study were fed mineralized milk; one group was maintained at a relative humidity of 12 to 24 per cent, the other group at 65 to 75 per cent. Tissues were taken from each group daily over a three-week period. Animals maintained at low humidity showed dryness of the skin of the paws after two days on the experimental diet and cracking of the skin by the third to fifth day. This was followed by scaling reaching maximal intensity by the tenth to twelfth day, with spontaneous recovery observed two to three weeks later. Rats maintained at high relative humidity did not show outward evidence of a lesion. In contrast to the pronounced outward appearing differences of the two groups, no differences in microscopic appearance of the skin were detected. The outstanding features of the histologic picture are the hyperkeratosis of the skin and the hyperkeratinization of the hair follicles.

R. A. Collins, M. Schreiber, and C. A. Elvehjem (*J. Nutrition* **49,** *589* (*1953*)) studied the influence of relative humidity

upon the dermatoses in vitamin A, riboflavin, pantothenic acid and pyridoxine deficiencies. These investigators used highly purified diets containing casein, sucrose, cottonseed oil and a salt mixture and also a suitable mixture of vitamins. Omission of one member of this vitamin mixture furnished the required deficient diet. Groups of animals were exposed to relative humidities of 5 to 10 per cent, 30 per cent, 50 per cent and 90 per cent. Growth rates of rats fed the complete diet were not affected by relative humidity. On pyridoxine-deficient or low pyridoxine diets growth seemed to be somewhat improved when the animals were maintained at 90 per cent relative humidity. Average daily water intake was greater when the animals were exposed to relative humidities of 5 to 10 per cent. Regardless of the diet fed, a scaly dermatosis developed in all animals maintained at 5 to 10 per cent relative humidity. Lesions appeared similar to those described for rats on lactose diets and maintained at low humidity. Again, spontaneous recovery was observed when the period of most rapid growth was completed. At 30 per cent relative humidity dermatosis occurred only in animals receiving the deficient diets, while those receiving the complete rations remained normal. Animals exposed to an atmosphere of 90 per cent relative humidity developed a red pigmentation of the fur about the head and body. This staining was least pronounced in the rats receiving the complete diet. The red fluorescence of the pigment under ultraviolet light was similar to that of some porphyrin pigments.

These experiments suggest that further investigation of the effect of relative humidity on the appearance of deficiency symptoms would be highly profitable. Should the results of the Wisconsin investigators be confirmed in other laboratories, it would be imperative in nutritional investigations to control not only temperature but relative humidity as well.

METABOLIC BLOCKS IN DIABETES

Although the importance of dietary management in diabetes mellitus is widely recognized and appreciated, there is still some difference of opinion as to the dietary regimen of choice. One cause of this disagreement is the lack of knowledge concerning the precise metabolic function of the hormone insulin. Consequently, adequate dietary regimens for the diabetic patient have had to be arrived at empirically. Against this background, a paper by I. L. Chaikoff (*Harvey Lectures* **47,** *99 (1951/52)*) is of particular interest since it draws together a significant portion of recent experimental work in this field.

Chaikoff points once again to the early, widely held concept that the conversion of glucose into fat was a means of disposing of excess calories. Although some experimental evidence showed rather early that this concept was too limited, it was not until the publication of DeW. Stetten and G. E. Boxer (*J. Biol. Chem.* **155,** *231 (1944)*) that real emphasis was placed on lipogenesis from carbohydrates as a normal pathway of carbohydrate metabolism. E. J. Masoro, I. L. Chaikoff, and W. G. Dauben (*Ibid.* **179,** *1117 (1949)*) reported experiments in which mice were fed a high carbohydrate diet containing glucose evenly labeled with C^{14}. After forty-eight hours of this regimen, the carcass fatty acids contained 13 per cent of all the administered C^{14}. During this two-day period, 76 per cent of the injected C^{14} was excreted as carbon dioxide. The specific activity of the fatty acids isolated (specific activity expressed as counts per minute per milligram of fatty acid) was far greater for liver than for carcass, indicating again that the liver is particularly

concerned with lipogenesis from carbohydrate. However, evidence was presented in this paper which showed that the eviscerated rat was also capable of incorporating C^{14} from glucose into fatty acids. Using liver and kidney slices as well as rat diaphragm, S. S. Chernick, Masoro, and Chaikoff (*Proc. Soc. Exp. Biol. Med.* **73,** *348 (1950)*) were able to show that liver is some ten times as active as kidney and diaphragm in incorporating C^{14} from glucose into fatty acids.

E. J. Masoro, I. L. Chaikoff, S. S. Chernick, and J. M. Felts (*J. Biol. Chem.* **185,** *845 (1950)*) reported on the effects of fasting and of certain dietary manipulations on the rate of lipogenesis from carbohydrate in liver slices. Fasting or the feeding of a high fat diet was found to reduce fat formation under these conditions. In this connection it is interesting to note that others have demonstrated that fasting and fat feeding reduces glucose tolerance, lowers glycogen synthesis in isolated diaphragm, diminishes the insulin content of pancreas, and lowers glucose absorption from the alimentary tract. I. Lyon, M. S. Masri, and Chaikoff (*Ibid.* **196,** *25 (1952)*) showed that a twelve-hour fast reduced, while an eighteen-hour fast almost completely abolished, the incorporation of acetate into fatty acids by liver slices. Administration of a single dose of glucose following prolonged fasting restored the ability to convert acetate into fatty acid. This effect was felt for at least eighteen hours after glucose feeding.

Having established these basic facts about lipogenesis in isolated liver slices, Chaikoff's group then went on to study carbon dioxide production and fatty acid synthesis in alloxan-diabetic rats. Liver slices taken from diabetic animals and incubated with C^{14}-labeled glucose showed markedly reduced carbon dioxide formation and almost total abolition of fatty acid synthesis. This work of S. S. Chernick, I. L. Chaikoff, E. J. Masoro, and E. Isaeff (*J. Biol. Chem.* **186,** *527 (1950)*) was in general agreement with the report of DeW. Stetten and G. E. Boxer (*Ibid.* **156,** *271 (1944)*). Further analysis of this problem by Chaikoff's group showed that liver slices incubated in a medium containing C^{14}-labeled fructose yielded the same amount of carbon dioxide whether the slices were taken from normal or from diabetic animals. On the other hand, fatty acid synthesis from fructose was markedly reduced in the diabetic livers. The fact that liver slices from diabetic rats showed diminished carbon dioxide formation from glucose while converting fructose into carbon dioxide at the same rate as liver slices from normal animals would seem to suggest that the absence of insulin either reduces the phosphorylation of glucose to glucose-6-phosphate or else diminishes the conversion of glucose-6-phosphate to fructose-6-phosphate (the latter possibility was ruled out when it was demonstrated that the phosphohexoseisomerase activities of normal and diabetic liver were identical).

A second metabolic block due to the absence of insulin is suggested by the fact that glucose, fructose and acetate are converted to fatty acids at a slower rate in the diabetic liver. Feeding a high fructose diet for three days before killing the animal completely restored the ability of liver slices to convert acetate into fatty acids in the diabetic animal. The fructose treatment, however, had no effect on the oxidation of glucose to carbon dioxide. From this it might be concluded that retardation of lipogenesis from acetate is not the primary metabolic block attributable to the absence of insulin, but that it is rather a secondary effect of a block in glucose utilization. It would appear from this that effective glucose oxidation must be maintained in order to retain the integrity of the enzyme systems responsible for lipogenesis from acetate. Further experiments on the role of insulin in acetate disposition in the liver showed this hormone to have a directive influence. In its presence more fatty acid was synthe-

sized from acetate while relatively less carbon dioxide was produced. In the absence of insulin, lipogenesis diminished while carbon dioxide production correspondingly increased (J. M. Felts, I. L. Chaikoff, and M. J. Osborn, *J. Biol. Chem.* **193,** *557 (1951)*). Evidence has also been presented showing that insulin inhibits the conversion of fatty acids or at least of acetate into glucose.

THE ACTION OF VITAMIN B_6 ANTAGONISTS IN MICROORGANISMS

Although 4-desoxypyridoxine acts as a biologic antagonist to vitamin B_6 in higher animals, its effect on microorganisms has been varied. In certain microorganisms a vitamin B_6-like action on growth has been observed, whereas, as shown by G. J. Martin, S. Avakian, and J. Moss (*J. Biol. Chem.* **174,** *495 (1948)*), 4-desoxypyridoxine inhibits the growth of *Saccharomyces cerevisiae*. The biologic activity of certain pyridoxine analogues has been studied further by J. C. Rabinowitz and E. E. Snell (*Arch. Biochem.* **43,** *399 (1953)*) in a variety of microbial species. Four organisms which are able to grow in the absence of vitamin B_6 were grown in the presence of 4-desoxypyridoxine. No inhibition of the growth of any of these microorganisms occurred.

Six organisms which require vitamin B_6 for growth were tested in the presence of 4-desoxypyridoxine. Inhibition of growth occurred in three of the organisms tested, *S. carlsbergensis*, *S. cerevisiae* and *Neurospora sitophila*. The response to *S. carlsbergensis* is of special interest, because this organism requires vitamin B_6 only in the presence of thiamine. When thiamine was absent from the mediums good growth was obtained without added vitamin B_6. In the absence of thiamine over one hundred and fifty times as much 4-desoxypyridoxine was required to inhibit growth as compared to the amount of the analogue required when both thiamine and pyridoxal hydrochloride were present in the mediums. When vitamin B_6 was provided in the absence of thiamine, conditions under which the organism can synthesize the vitamin but is not required to do so, an intermediate sensitivity to the inhibitor was found.

A similar situation exists in the case of *N. sitophila*. Growth occurs when this organism is provided with a medium containing ammonium ions as the nitrogen source at pH 6.0 or higher, in the absence of vitamin B_6. At lower pH values vitamin B_6 is required for growth. Under conditions in which added vitamin B_6 was required for growth, 4-desoxypyridoxine proved to be a potent inhibitor. When the mediums permitted the synthesis of vitamin B_6 by the organism, no inhibition was obtained by the addition of 4-desoxypyridoxine.

The effectiveness of the different forms of vitamin B_6 in counteracting the inhibition of growth produced by 4-desoxypyridoxine was studied. It was found that in the case of *S. carlsbergensis* pyridoxine was most active. Pyridoxamine and pyridoxal were less active. In the reversal of inhibition of 4-desoxypyridoxine in *N. sitophila* pyridoxine was slightly more active than pyridoxal. Pyridoxamine was considerably less active.

In interpreting the above results the authors have suggested that 4-desoxypyridoxine may act primarily by preventing absorption of vitamin B_6 into the cell. If the inhibition does actually occur within the cells the experiments suggest that endogenously synthesized vitamin B_6 is never released in the free form and thus subject to inhibition by the antivitamin.

The action of several different vitamin B_6 antagonists was compared in a second paper (J. C. Rabinowitz and E. E. Snell, *Arch. Biochem.* **43,** *408 (1953)*). When tested for

growth inhibition on *S. carlsbergensis* the order of decreasing effectiveness was found to be omega-methylpyridoxine, 4-desoxypyridoxine, 5-desoxypyridoxine, 5-desoxypyridoxal, 5-desoxypyridoxamine. The inhibition of these antivitamins was most effectively counteracted by pyridoxine. Pyridoxamine was least effective and pyridoxal was intermediate.

The inhibition of growth of *Streptococcus faecalis* produced by the 5-desoxy analogues proved to be different from that of 4-desoxypyridoxine. Whereas 5-desoxypyridoxamine was an effective antagonist and 5-desoxypyridoxal was somewhat less active, the other components tested were ineffective. Pyridoxamine was most effective in overcoming the inhibition of 5-desoxypyridoxamine. The sole metabolic reaction for which an external source of vitamin B_6 is required by *S. faecalis*, grown on the mediums employed, is the synthesis of alanine. Good growth occurs in the absence of the vitamin when exogenous alanine is provided. It was found that in an alanine-supplemented medium 5-desoxypyridoxal and 5-desoxypyridoxamine no longer inhibit growth of *S. faecalis*.

The effect of adding more than one inhibitor on the growth of *S. carlsbergensis* was studied. The inhibition of growth produced by 4-desoxypyridoxine and omega-methylpyridoxine was additive, suggesting that the same reaction was being inhibited by these analogues. The inhibitions produced by 4-desoxypyridoxine and 5-desoxypyridoxal were not additive. This observation was interpreted as suggesting independent inhibitory mechanisms.

The conclusion which may be drawn from these studies is that there may be a number of mechanisms by which analogues of vitamin B_6 act. One possible mechanism has been discussed previously (*Nutrition Reviews* **8,** *28 (1950)*). *S. faecalis* preparations phosphorylate 4-desoxypyridoxine, and this coenzyme analogue may then interfere with the action of pyridoxal phosphate in reacting with the apoenzyme of tyrosine carboxylase. Despite the proven interference with this enzyme system, growth of *S. faecalis* is not inhibited by 4-desoxypyridoxine. An entirely different type of antagonism exists for the 5-desoxy analogues because phosphorylation at the 5 position is blocked. The authors suggest that these analogues may interfere with the absorption of vitamin B_6 by the cell or else inhibit the phosphorylation of vitamin B_6.

THE DIABETOGENIC ACTIVITY OF PITUITARY EXTRACTS

The relationship of the diabetogenic activity of pituitary extracts to the pituitary growth hormone remains a difficult problem in experimental endocrinology and is one of importance to those interested in protein nutrition. Despite the fact that most observers have been able to demonstrate diabetogenic activity in growth hormone preparations when injected into suitable test animals, a number of divergent experimental findings remain to be explained. For instance, the ratio of diabetogenic to growth activity of certain crude pituitary extracts has been found to be variable by H. P. Marks and F. G. Young (*Lancet* **I,** *493 (1940)*) and suggests that the two metabolic activities may not depend on identical structures in the same molecule.

Support for the view that growth activity and diabetogenic activity may be separated has been provided by the observations of M. S. Raben and V. W. Westermeyer (*Proc. Soc. Exp. Biol. Med.* **80,** *83 (1952)*). These authors have prepared growth hormone by extraction of pituitary powders with glacial acetic acid and purification using oxycellulose. A growth hormone was obtained which the authors state was nondiabetogenic.

The latest of a series of papers on this problem has been published by E. Reid (*J. Endocrinol.* **9,** *210* (*1953*)). Pituitary preparations have been tested for diabetogenic activity (D) in intact cats and for growth activity (G) in mature female rats. The biologic activities of preparations tested were expressed as equivalent to a certain weight of a highly purified growth hormone standard. Some confirmation was obtained of the observed loss of diabetogenic activity of pituitary extracts treated according to the method of Marks and Young with respect to growth activity. It was noted, however, that there was a wide range of variation of the D/G ratios even of untreated extracts, and the decrease of diabetogenic activity was variable.

Experiments designed to determine what conditions in the extraction procedure determine the D/G ratio were not productive. Purification of diabetogenic fractions led to preparations whose principal component on ultracentrifugal study proved to sediment at a rate comparable to that of growth hormone. The D/G ratio of three pituitary extracts made according to Marks and Young was measured. Two of these extracts originally had a low D/G ratio whereas the ratio was high for the third extract. Isoelectric precipitation of these extracts gave a fraction rich in growth hormone. The D/G ratios of the derived fractions in each case proved to be nearly unity as compared to a purified growth hormone standard.

In a previous publication, E. Reid (*J. Endocrinol.* **8,** *50* (*1952*)) reported studies of the D/G ratio of growth hormone partially inactivated in several ways. Repeated precipitation, incubation with a proteolytic enzyme from *Bacillus subtilis*, incubation at pH 2.8, iodination and acetylation did not produce a significant alteration of D/G ratio. Further unsuccessful attempts to dissociate D/G activity of growth hormone by chemical means were recorded (Reid, *Ibid.* **9,** *210* (*1953*)). A preparation of purified bovine growth hormone was treated with oxidized cellulose under the conditions described by Raben and Westermeyer (*loc. cit.*). No significant change in the D/G ratio was produced by this treatment. A sample of pig growth hormone prepared by Raben and Westermeyer was found to have diabetogenic activity in proportion to growth activity similar to the bovine growth hormones previously tested.

The experiments reported above support the view that the variability of the D/G ratio of the crude extracts cannot be attributed to variability of the D/G ratio of the contained growth hormone. The most probable explanation of the observed facts is that crude extracts may contain variable amounts of other factors which might influence the diabetogenic or growth activity of growth hormone. Considerable evidence for such a view exists. Thyrotropic hormone increases the apparent growth activity of growth hormone, whereas ACTH decreases growth under certain conditions. E. Reid (*J. Endocrinol.* **9,** *185* (*1953*)) has found that the diabetogenic activity of growth hormone is enhanced by the additional administration of corticotropin to intact cats. The administration of corticotropin by itself did not produce diabetes in intact cats. When different ACTH preparations were tested for their ability to enhance the diabetogenic activity of growth hormone, it was found that the diabetogenic augmentation was not proportional to the potency of these preparations in Sayers' test (M. A. Sayers, G. Sayers, and L. A. Woodbury, *Endocrinology* **42,** *379* (*1948*)).

Direct proof that the variations in D/G ratios can be explained on the basis of partial inactivation or augmentation of ACTH and thyrotropic hormone in crude extracts has not been obtained. The problem of assaying ACTH in these extracts is complicated. In the usual Sayers' test little ACTH has been found to be present. However, these extracts are effective in restoring the weight of the adrenal glands of hypophysectomized rats (M. Stack-Dunne and F. G. Young, *J. Endocrinol.* **7,** *lxvi* (*1951*)).

In conclusion, the papers reviewed sup-

port the view that diabetogenic activity is intrinsic in the growth hormone molecule. The separation of diabetogenic and growth activity claimed by Raben and Westermeyer has not been confirmed by Reid. Variations in diabetogenic potency in comparison to growth activity of crude pituitary extracts are probably due to alterations of non-growth hormone components which modify the action of this hormone.

ANTIBACTERIALS AND GROWTH

It has been demonstrated by several laboratories that the addition of minimal amounts of certain antibiotics to an otherwise adequate diet will significantly increase the growth rate of a variety of animals (*Nutrition Reviews* **9,** *359* (*1951*)). The observation has proved of such practical value to husbandrymen that antimicrobial products are now marketed to farmers throughout the nation for this purpose.

Information regarding the mechanisms by which such increased growth is brought about, however, is fragmentary. All of the agents used have a relatively broad spectrum of antimicrobial action, and inhibit many of the aerobic organisms found in the intestinal lumen. That they will also suppress or destroy anaerobic forms is suggested by the data of J. S. Kiser, G. C. de Mello, D. H. Reichard, and J. H. Williams (*J. Inf. Dis.* **90,** *76* (*1952*)), who demonstrated a significant action of aureomycin and other compounds on certain enteric clostridia. Inhibition of *Clostridium perfringens* in the intestinal tract has been suggested as a possible explanation of the growth-promoting effects of penicillin and terramycin in the turkey (J. M. Sieburth *et al.*, *Proc. Soc. Exp. Biol. Med.* **76,** *15* (*1951*)), and several studies reported at the Lobund Institute Colloquium at Notre Dame in 1952 offered strong evidence that the growth-promoting action is dependent on the antimicrobial action. The growth of germ-free birds is not increased by antibiotics.

Recent studies offer further evidence that antibiotics produce increased weight gains, and also show that this faculty extends to certain penicillin products as well as to two arsenicals, and to dried whey (J. F. Elam *et al.*, *J. Nutrition* **49,** *307* (*1953*)). In each instance where a factor fed (or injected) resulted in a significant growth increase, there occurred a profound depression of fecal clostridia (save for dried whey). New Hampshire chicks were separated into two groups, kept in cages with screened, raised floors and given a supplemented diet. Antibiotics were fed in the proportion of 10 g. per ton of feed, except that penicillin was given at a level of 4 g. per ton of feed. In a second experiment, using combinations of agents, the total amount of antibiotics fed never exceeded 10 g. per ton of feed. Penicillin and penicillin inactivated by autoclaving were injected in amounts of 1.2 mg. per bird per week.

The feces were collected weekly and cultures were made for anaerobes, primarily the genus *Clostridium*. Addition to the diet of penicillin, aureomycin, bacitracin, or combinations of penicillin and aureomycin, and combinations of bacitracin and penicillin, caused growth increases of similar extent in the birds. The addition of a 3 per cent dried whey to the basal diet gave an increase in growth rate comparable to that obtained with the antibiotics without the whey. Further increase in growth, as compared to that produced by the whey diet, was obtained when the whey was supplemented with penicillin by mouth or by injection, or by supplements of penicillin plus aureomycin or bacitracin. This suggested a synergistic relationship with respect to growth stimulation between penicillin and the dried whey.

Penicillin, given either orally or by injection, injections of inactivated penicillin (but not oral inactivated penicillin), com-

binations of penicillin and aureomycin, and combinations of bacitracin and penicillin, produced a very significant decrease in the total number of fecal clostridia. Inactivated penicillin fed by mouth also decreased fecal clostridia, but much less so than the other agents, and failed to increase the growth of the birds. It was thought that the inactivated penicillin, when injected into the bird's tissues, was reactivated in some manner, but was not so influenced when fed by mouth. When dried whey alone was added to the diet, a decrease of clostridia in the feces was often found, but this was not quite statistically significant. Significant decreases in fecal clostridia were found when antibiotics were added to the 3 per cent dried whey diet. The close relationship between the administration of an antibiotic, the reduction of the number of fecal clostridia, and the accelerated growth rate fits well with studies where inhibition of *Clostridium perfringens* in the turkey is related to the growth-stimulating property of penicillin and terramycin.

It is of interest to note that the anticlostridial action of the antibiotics, or more properly of aureomycin, is related not only to growth-promoting factors, for recent evidence indicates the feeding of this agent to the point where feces are relatively free of clostridia is associated with a tremendous augmentation of the animal's resistance to shock (H. A. Frank *et al.*, *Am. J. Physiol.* **168,** *430 (1952)*). These studies have demonstrated that dogs subjected to a standardized procedure of hemorrhage show a mortality of 88 per cent. On the other hand, when animals are pretreated with aureomycin for periods of from six to twelve days, to the point where the feces are free of clostridia, the mortality rate is reduced to approximately 12 per cent. This increased survival rate is associated not only with a relative absence of fecal clostridia, but with an inability to demonstrate the usual increase in bacterial content of the liver in the animal dying of hemorrhagic shock. These findings imply that this toxin-producing bacterium, whether by action within the viscera or the liver, and possibly in other areas of the splanchnic region, may have a role in the phenomenon of "irreversibility" caused by hemorrhagic shock.

These studies, coming from rather widely separated areas of experimental physiology, add evidence to the concept that the enteric bacteria may be a hazard to the well-being of the organism, even though in a "normal" state of health their unfavorable action is not apparent. The findings, in regard both to the improvement of growth when small amounts of these antimicrobials are fed, and to their protective action against a standardized shock procedure, would seem to justify the further experimental studies of antimicrobial factors in an attempt to evaluate possible roles of bacteria and bacterial products in other phenomena, perhaps in the so-called "degenerative diseases" of animals and man.

NOTES

Letter to the Editor

Dear Sir:

May I call your attention to an error in the July 1953 issue of *Nutrition Reviews*, page 211, column 2, paragraph 3, line 8, in which it is stated that "sardine meal in the diet produced intense fishiness in the cooked carcass." A review of the original article shows that fishiness was found after feeding 2 per cent sardine oil, not the meal.

It might be mentioned that practical turkey rations contain about 3 per cent fish meal, but the sardine oil added seldom exceeds 0.35 per cent.

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American Board of Nutrition

The American Board of Nutrition will hold certifying examinations in Atlantic City, New Jersey, during April 1954. Completed applications of persons wishing to be considered for certification should be in the office of the Secretary not later than February 1, 1954. Application forms may be obtained from the Secretary, Otto A. Bessey, Department of Biochemistry and Nutrition, The University of Texas School of Medicine, Galveston, Texas.

Nutrition for Airmen and Industrial Workers

"Human Factors in Air Transportation; Occupational Health and Safety" (*McGraw-Hill Book Company, Inc., New York (1953)*) is the title of a new book by Dr. Ross A. McFarland, Associate Professor of Industrial Hygiene, Harvard School of Public Health, in which are found several sections on the nutritional problems of flying and of industry in general. It is stated that "among those variables which may affect the over-all efficiency of the airman, there are few more important than food. An improper diet along with overeating, constipation, and other digestive irregularities produce both mental and physical lethargy. An airman may even experience weakness, dizziness, headaches, and various other physical ailments if good dietary habits are not observed while on flight duty."

The significant role played by food and nutrition in air transportation and other industries is illustrated by the range of topics discussed. These include: industrial nutrition programs applied to airline personnel and other workers, sanitary supervision of food in flight and at foreign and domestic airports, in-flight food service, survival rations for crew and passengers, studies of nutritional status of air personnel, factors influencing digestion in flight, increasing altitude tolerance by diet, influence of constipation and diarrhea on efficiency.

Milk Diets and Vitamin B_{12}

In spite of the discovery twenty-five years ago (E. B. Hart, H. Steenbock, J. Waddell, and C. A. Elvehjem *J. Biol. Chem.* **77,** *797 (1928)*) that iron and copper would cure anemia in animals fed a diet of cow's milk, the effectiveness of these minerals in alleviating deficiency symptoms in animals fed a diet of goat's milk was not settled. Recently, it was reported that the inferior growth of rats fed a mineralized goat's milk was due to a deficiency of vitamin B_{12} (R. A. Collins, M. Schreiber, Elvehjem, and Hart, *J. Nutrition* **49,** *485 (1953)*). Thus was verified a postulate made about twenty years ago by European workers that goat's milk may be deficient in Castle's "extrinsic factor" or vitamin B_{12}. Collins, A. E. Harper, Schreiber, and Elvehjem (*Ibid.* **43,** *313 (1951)*) found that goat's milk contained 0.15 micrograms of vitamin B_{12} per liter, while a much higher level of this vitamin was present in cow's milk (3 to 4 micrograms per liter).

CLINICAL NUTRITION BRIEFS

"Review of the feeding of extreme premature infants at the Chicago Lying-In Hospital revealed that the 45 per cent incidence of retrolental fibroplasia occurring in 1946–1949 was associated with formula feedings while previous and subsequent experiences with human milk or relatively dilute cow's milk formulas led to an incidence of only 7 per cent." (*Nutrition Reviews* **11,** *103* (*1953*), Sodium Restriction and Retrolental Fibroplasia.)

"These results indicate that in the gouty individual a larger than normal fraction of dietary glycine is utilized for synthesis of uric acid. On the basis of the few studies available this metabolic defect is probably exaggerated by a high protein diet. With this knowledge dietary intake can be seen to be highly contributory to the gout syndrome, but the inherent metabolic defect is not correctable by dietary measures alone." (*Nutrition Reviews* **11,** *107* (*1953*), Diet and the Metabolic Defect in Gout.)

"As a result of this observation, J. H. van de Kamer and H. A. Weijers studied the role of wheat in the diet of celiac patients. Their findings were dramatic. They observed that whenever the diet of such patients contained wheat, even in very small quantities, the patients became pale, the appetite disappeared, weight was lost, diarrhea occurred and steatorrhea increased. As soon as the wheat flour was left out of the diet all the unfavorable symptoms became less marked or disappeared entirely and the general condition of the child improved." (*Nutrition Reviews* **11,** *199* (*1953*), Wheat Gluten and the Celiac Syndrome.)

"In the men who were initially markedly overweight (expected mortality of 179 per cent), mortality of those who reduced their weight was 109 per cent. In women similar trends were observed." (*Nutrition Reviews* **11,** *144* (*1953*), Obesity, Weight Reduction and Longevity.)

"It is concluded that the low fat diet used in this study of the treatment of multiple sclerosis was of considerable effectiveness." (*Nutrition Reviews* **11,** *171* (*1953*), Low Fat Diet in Treatment of Multiple Sclerosis.)

"These studies indicate that fat absorption as it is measured by vitamin A tolerance may be adversely affected in colitis, but amino acid absorption is less often affected while carbohydrate absorption may be enhanced." (*Nutrition Reviews* **11,** *237* (*1953*), Intestinal Motility and Absorption.)